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**(54) Title:** DETECTION OF FERMENTATION-RELATED MICROORGANISMS

**(57) Abstract**

Unique DNA sequences are provided which are useful in identifying different fermentation-related microorganisms, such as those involved in fermentations. These unique DNA sequences can be used to provide oligonucleotide primers in PCR based analysis for the identification of fermentation-related microorganisms. The DNA sequences of the present invention include the internal transcribed spacer (ITS) of the ribosomal RNA gene regions of particular fermentation-related microorganisms, as well as oligonucleotide primers which are derived from these regions which are capable of identifying the particular microorganism.

ITS

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## DETECTION OF FERMENTATION-RELATED MICROORGANISMS

### Field of the Invention

The present invention relates to assays to detect fermentation-related microorganisms.

### 5 Description of the Related Art

In traditional wine-making, the indigenous yeasts ferment the grape must. Most modern wine-makers, however, inoculate with a pure culture of a selected yeast strain to ensure a rapid, reliable and predictable fermentation. It is thought that indigenous yeasts are suppressed by the competitive effect of  
10 addition of a high-density monoculture, but some evidence suggests that indigenous yeast can still participate in the fermentation. A range of commercial yeasts with different wine-making characteristics is available, and a number of those yeast strains may be used in a single winery. Furthermore, unwanted microorganisms may be present which lead to spoilage. Therefore,  
15 there is a need for a rapid, simple and accurate method for identifying microorganisms in starter cultures and fermentations.

Past techniques for detecting and identifying fermentation-related microorganisms, especially yeast, include colony morphology, fermentation performance, sugar fermentation tests, tolerance to various stresses (e.g.,  
20 ethanol tolerance in brewing), phenotypes with functional relevance (e.g., flocculation in brewing), nutritional requirements (e.g., oxygen), and resistance and sensitivity levels of cycloheximide. These methods, however, have numerous disadvantages, including lengthy analysis periods, inability to differentiate, e.g., different strains of yeast, and lack of reproducibility.

Recent developments in molecular biology and protein chemistry have provided new methods for identifying microorganisms, including DNA restriction fragment length polymorphisms, protein electrophoresis patterns and chromosome fingerprinting. Such techniques have been used for identifying fermentation-related microorganisms. See, for example, Casey et al, *Journal of the American Society of Brewing Chemists*, 48(3):100-106, 1990; Degre et al, *American Journal of Enology and Viticulture*, 40(4):309-315, 1989; Guillaumon et al, *Systematic and Applied Microbiology*, 19:122-132, 1992; Hoeben et al, *Current Genetics*, 10:371-379, 1986; Mozina et al, *Letters in Applied Microbiology*, 24(4):311-315, 1997; Paffetti et al, *Research Microbiology*, 146:587-594, 1995; Panchal et al, *Journal of the Institute of Brewing*, 93:325-327, 1987; Querol et al, *Systematic and Applied Microbiology*, 15:439-446, 1992; Vezinhet et al, *Applied Microbiology and Biotechnology*, 32:568-571, 1990; and Vezinhet et al, *American Journal of Enology and Viticulture*, 43(1):83-86, 1992.

Polymerase chain reaction (PCR)-based techniques have also been used to detect fermentation-related microorganisms. See, for example, DeBarros Lopes et al, *Applied and Environmental Microbiology*, 62(12):4514-4520, 1996; Fell, *Molecular Marine Biology and Biotechnology*, 2(3):174-180, 1993; Fell, *Journal of Industrial Microbiology*, 14(6):475-477, 1995; Ibeas et al, *Applied and Environmental Microbiology*, 62(3):998-1003, 1996; Lavallee et al, *American Journal of Enology and Viticulture*, 45(1):86-91, 1994; Lieckfeldt et al, *Journal of Basic Microbiology*, 33(6):413-425, 1993; and Ness et al, *J. Sci. Food Agric.*, 62:89-94, 1993.

Ribosomal genes are suitable for use as molecular probe targets because of their high copy number. Non-transcribed and transcribed spacer sequences associated with ribosomal genes are usually poorly conserved and, thus, are

advantageously used as target sequences for the detection of recent evolutionary divergence. Fungal rRNA genes are organized in units. Each unit encodes mature subunits of 18S, 5.8S, and 28S rRNA. The internal transcribed spacer (ITS) region lies between the 18S and 28S rRNA genes and contains two  
5 variable non-coding spacers (referred to as ITS1 and ITS2) and the 5.8S rRNA gene (White et al., 1990; In: *PCR Protocols*; Eds.: Innes et al.; pages 315-322). In addition, the transcriptional units are separated by non-transcribed spacer sequences (NTSs). The ITS and NTS sequences are particularly suitable for the detection of different fungal pathogens.

10 Kumeda et al (*Applied and Environmental Microbiology*, 62(8):2947-2952, 1996) describes use of PCR to amplify ribosomal DNA internal transcribed spacers in order to differentiate species of *Aspergillus* Section *Flavi*. The ITS1-5.8S-ITS2 region was amplified using universal primers, and the PCR product analyzed by the principle of single-strand conformation  
15 polymorphism. In addition, Gardes et al (In: *Methods in Molecular Biology*, Vol. 50: *Species Diagnostics Protocols: PCR and Other Nucleic Acid Methods*, Ed. J.P. Clapp, Humana Press, Totowa, NJ, (1996) pp. 177-186) describes restriction fragment length polymorphism (RFLP) analysis of fungal ITS regions amplified by PCR.

20 The PCR amplification of fungal ITS has also been described using other than universal primers. These methods allow for more specificity in identifying classes of fungi, or particular species of fungi. Thus, Gardes and Bruns (*Molecular Ecology*, 2:113-118, 1993) identified ITS primers which allow differentiation of DNA from basidiomycetes against ascomycete DNA.  
25 Identification of specific species has been observed using PCR primers directed to unique sequences in the ITS1 and/or ITS2 regions of fungal pathogens. See, for example, Hamelin et al, *Applied and Environmental Microbiology*, 62(11):4026-4031, 1996; Mazzola et al, *Phytopathology*, 86(4):354-360, 1996;

O'Gorman et al, *Canadian Journal of Botany*, 72:342-346, 1994; and U.S. Patent No. 5,585,238 to Ligon et al.

The present invention addresses the problem of detecting and identifying fermentation-related microorganisms by PCR-based techniques.

5     **Summary of the Invention**

          The present invention is directed to the identification of different fermentation-related microorganisms, particularly those involved in the production of wine. The present invention provides DNA sequences which exhibit variability between different fermentation-related microorganisms. In particular, the present invention identifies regions of DNA sequence located in the internal transcribed spacer (ITS) of the ribosomal RNA gene regions of various fermentation-related microorganisms. Primers derived from the ITS can be used in polymerase chain reaction (PCR) based diagnostic assays to determine the presence or absence of specific fermentation-related microorganisms, including those involved in the production of wine. The primers can also be used as molecular probes to detect the presence of target DNA.

          Thus, in one aspect, the present invention provides an isolated double stranded nucleic acid of the full length ITS1 or ITS2 region of a fermentation-related microorganism. More particularly, the DNA sequence is selected from among Sequence ID NOS: 13 to 36.

          In another aspect, the present invention provides an oligonucleotide primer for identification of a fermentation-related microorganism, wherein the primer is a divergent portion of the ITS1 or ITS2 region of a fermentation-related microorganism. More particularly, the oligonucleotide primer is selected from among Sequence ID NOS: 65 to 98. Furthermore, the oligonucleotide primers may be selected from among sequences which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98,

primers which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64, and primers of 10 nucleotide bases or longer which contain at least 5 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with from 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64. A pair of the foregoing oligonucleotide primers for use in the amplification-based detection of an ITS of a fermentation-related microorganism is also provided.

In yet another aspect, a method is provided for detection of a fermentation-related microorganism which comprises: (a) obtaining DNA from a fungal culture or colony isolated from a fermentation, or from an organism present in a fermentation beverage; (b) amplifying a part of the ITS of the fermentation-related microorganism using the DNA as a template in a polymerase chain reaction with the aforementioned oligonucleotide primers; and (c) visualizing the amplified part of the ITS sequence to determine whether the fermentation-related microorganism is present.

In still another aspect, kits are provided which are useful in detecting fermentation-related microorganisms.

**Brief Description of the Sequences in the Sequence Listing:**

- |    |              |  |
|----|--------------|--|
| 20 | SEQ ID NO: 1 | DNA sequence for the internal transcribed spacer of <i>Saccharomyces cerevisiae</i> and <i>Saccharomyces bayanus</i> . |
|    | SEQ ID NO: 2 | DNA sequence for the internal transcribed spacer of <i>Saccharomyces ludwigii</i> .                                    |
|    | SEQ ID NO: 3 | DNA sequence for the internal transcribed spacer of <i>Dekkera bruxellensis</i> .                                      |
| 25 | SEQ ID NO: 4 | DNA sequence for the internal transcribed spacer of <i>Dekkera intermedia</i> .  |

- SEQ ID NO: 5 DNA sequence for the internal transcribed spacer of *Botrytis cinerea*.
- SEQ ID NO: 6 DNA sequence for the internal transcribed spacer of *Penicillium crustosum*.
- 5 SEQ ID NO: 7 DNA sequence for the internal transcribed spacer of *Penicillium expansum*.
- SEQ ID NO: 8 DNA sequence for the internal transcribed spacer of *Hanseniaspora guilliermondii*.
- SEQ ID NO: 9 DNA sequence for the internal transcribed spacer of *Debaryomyces carsonii*.
- 10 SEQ ID NO: 10 DNA sequence for the internal transcribed spacer of *Pichia anomala*.
- SEQ ID NO: 11 DNA sequence for the internal transcribed spacer of *Pichia kluyveri*.
- 15 SEQ ID NO: 12 DNA sequence for the internal transcribed spacer of *Candida krusei*.
- SEQ ID NO: 13 DNA sequence for the ITS1 of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.
- SEQ ID NO: 14 DNA sequence for the ITS2 of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.
- 20 SEQ ID NO: 15 DNA sequence for the ITS1 of *Saccharomycodes ludwigii*.
- SEQ ID NO: 16 DNA sequence for the ITS2 of *Saccharomycodes ludwigii*.
- 25 SEQ ID NO: 17 DNA sequence for the ITS1 of *Dekkera bruxellensis*.
- SEQ ID NO: 18 DNA sequence for the ITS2 of *Dekkera bruxellensis*.
- SEQ ID NO: 19 DNA sequence for the ITS1 of *Dekkera intermedia*.
- SEQ ID NO: 20 DNA sequence for the ITS2 of *Dekkera intermedia*.
- SEQ ID NO: 21 DNA sequence for the ITS1 of *Botrytis cinerea*.



	SEQ ID NO: 22	DNA sequence for the ITS2 of <i>Botrytis cinerea</i> .
	SEQ ID NO: 23	DNA sequence for the ITS1 of <i>Penicillium crustosum</i> .
	SEQ ID NO: 24	DNA sequence for the ITS2 of <i>Penicillium crustosum</i> .
	SEQ ID NO: 25	DNA sequence for the ITS1 of <i>Penicillium expansum</i> .
5	SEQ ID NO: 26	DNA sequence for the ITS2 of <i>Penicillium expansum</i> .
	SEQ ID NO: 27	DNA sequence for the ITS1 of <i>Hanseniaspora guilliermondii</i> .
	SEQ ID NO: 28	DNA sequence for the ITS2 of <i>Hanseniaspora guilliermondii</i> .
10	SEQ ID NO: 29	DNA sequence for the ITS1 of <i>Debaryomyces carsonii</i> .
	SEQ ID NO: 30	DNA sequence for the ITS2 of <i>Debaryomyces carsonii</i> .
	SEQ ID NO: 31	DNA sequence for the ITS1 of <i>Pichia anomala</i> .
	SEQ ID NO: 32	DNA sequence for the ITS2 of <i>Pichia anomala</i> .
	SEQ ID NO: 33	DNA sequence for the ITS1 of <i>Pichia kluyveri</i> .
15	SEQ ID NO: 34	DNA sequence for the ITS2 of <i>Pichia kluyveri</i> .
	SEQ ID NO: 35	DNA sequence for the ITS1 of <i>Candida krusei</i> .
	SEQ ID NO: 36	DNA sequence for the ITS2 of <i>Candida krusei</i> .
	SEQ ID NO: 37	Oligonucleotide Sequence SXUITS1a.
	SEQ ID NO: 38	Oligonucleotide Sequence SXLITS2a.
20	SEQ ID NO: 39	Oligonucleotide Sequence SXUITS1b.
	SEQ ID NO: 40	Oligonucleotide Sequence SXLITS2b.
	SEQ ID NO: 41	Oligonucleotide Sequence SLUITS1a.
	SEQ ID NO: 42	Oligonucleotide Sequence SLLITS2a.
	SEQ ID NO: 43	Oligonucleotide Sequence SLUITS1b.
25	SEQ ID NO: 44	Oligonucleotide Sequence SLLITS2b.
	SEQ ID NO: 45	Oligonucleotide Sequence BRUITS1a.
	SEQ ID NO: 46	Oligonucleotide Sequence BRLITS2.
	SEQ ID NO: 47	Oligonucleotide Sequence BRUITS1b.
	SEQ ID NO: 48	Oligonucleotide Sequence BCUITS1a.

	SEQ ID NO: 49	Oligonucleotide Sequence BCLITS2.
	SEQ ID NO: 50	Oligonucleotide Sequence BCUITS1b.
	SEQ ID NO: 51	Oligonucleotide Sequence PXUITS1a.
	SEQ ID NO: 52	Oligonucleotide Sequence PXLITS2a.
5	SEQ ID NO: 53	Oligonucleotide Sequence PXUITS1b.
	SEQ ID NO: 54	Oligonucleotide Sequence PXLITS2b.
	SEQ ID NO: 55	Oligonucleotide Sequence HGUITS1.
	SEQ ID NO: 56	Oligonucleotide Sequence HGLITS2.
	SEQ ID NO: 57	Oligonucleotide Sequence DXLITS2.
10	SEQ ID NO: 58	Oligonucleotide Sequence PAUITS1.
	SEQ ID NO: 59	Oligonucleotide Sequence PALITS2.
	SEQ ID NO: 60	Oligonucleotide Sequence PKLITS2.
	SEQ ID NO: 61	Oligonucleotide Sequence CKUITS1.
	SEQ ID NO: 62	Oligonucleotide Sequence CKLITS2.
15	SEQ ID NO: 63	Oligonucleotide Sequence SXU99.
	SEQ ID NO: 64	Oligonucleotide Sequence SXL658.
	SEQ ID NO: 65	Oligonucleotide Sequence SXU102.
	SEQ ID NO: 66	Oligonucleotide Sequence SXL661.
	SEQ ID NO: 67	Oligonucleotide Sequence SLU85.
20	SEQ ID NO: 68	Oligonucleotide Sequence SLL635.
	SEQ ID NO: 69	Oligonucleotide Sequence SLU88.
	SEQ ID NO: 70	Oligonucleotide Sequence SLL636.
	SEQ ID NO: 71	Oligonucleotide Sequence SLU136.
	SEQ ID NO: 72	Oligonucleotide Sequence SLL634.
25	SEQ ID NO: 73	Oligonucleotide Sequence BRU53A.
	SEQ ID NO: 74	Oligonucleotide Sequence BRU53B.
	SEQ ID NO: 75	Oligonucleotide Sequence BRU77.
	SEQ ID NO: 76	Oligonucleotide Sequence BRL339.
	SEQ ID NO: 77	Oligonucleotide Sequence BRL367.

	SEQ ID NO: 78	Oligonucleotide Sequence BRL390.
	SEQ ID NO: 79	Oligonucleotide Sequence BCU136.
	SEQ ID NO: 80	Oligonucleotide Sequence BCL393.
	SEQ ID NO: 81	Oligonucleotide Sequence BCU142.
5	SEQ ID NO: 82	Oligonucleotide Sequence PXU87.
	SEQ ID NO: 83	Oligonucleotide Sequence PXL495.
	SEQ ID NO: 84	Oligonucleotide Sequence PXU86.
	SEQ ID NO: 85	Oligonucleotide Sequence PXL482.
	SEQ ID NO: 86	Oligonucleotide Sequence PXL491.
10	SEQ ID NO: 87	Oligonucleotide Sequence HGU193.
	SEQ ID NO: 88	Oligonucleotide Sequence HGU231.
	SEQ ID NO: 89	Oligonucleotide Sequence HGL601.
	SEQ ID NO: 90	Oligonucleotide Sequence DXL447.
	SEQ ID NO: 91	Oligonucleotide Sequence DXL526.
15	SEQ ID NO: 92	Oligonucleotide Sequence PAU133.
	SEQ ID NO: 93	Oligonucleotide Sequence PAL451.
	SEQ ID NO: 94	Oligonucleotide Sequence PKL356.
	SEQ ID NO: 95	Oligonucleotide Sequence CKU104.
	SEQ ID NO: 96	Oligonucleotide Sequence CKL354.
20	SEQ ID NO: 97	Oligonucleotide Sequence ITS5.
	SEQ ID NO: 98	Oligonucleotide Sequence ITS4.

#### **Detailed Description Of The Invention**

The present invention provides unique DNA sequences which are useful in identifying fermentation-related microorganisms. These unique DNA sequences can be used as primers in PCR-based analysis for the identification of fermentation-related microorganisms, or as molecular probes to detect the presence of DNA from fermentation-related microorganisms. The DNA sequences of the present invention include the internal transcribed spacer (ITS)

of the ribosomal RNA gene regions of specific fermentation-related microorganisms, as well as primers that are derived from these regions which are capable of identifying the particular microorganism.

The DNA sequences of the invention are from the ITS of the ribosomal RNA gene region of fermentation-related microorganisms. However, the present invention is not limited to detecting the presence of the microorganisms in fermentation operations, *i.e.*, the invention can be used to detect the presence of such microorganisms from any source. There is variability in the ITS DNA sequences from different microorganisms. The ITS sequences can be aligned and compared. Primers can be designed based on regions within the ITS regions that contain the greatest differences in sequence among the fermentation-related microorganisms. The sequences and primers based on these sequences can be used to identify specific microorganisms.

DNA sequences of particular interest include ITS DNA sequences from *Saccharomyces* sp., especially *Saccharomyces cerevisiae* and *Saccharomyces bayanus*; *Saccharomyces* sp., especially *Saccharomyces ludwigii*; *Dekkera* sp., especially *Dekkera bruxellensis* and *Dekkera intermedia*; *Botrytis* sp., especially *Botrytis cinerea*; *Penicillium* sp., especially *Penicillium crustosum* and *Penicillium expansum*; *Hanseniaspora* sp., especially *Hanseniaspora guilliermondii*; *Debaryomyces* sp., especially *Debaryomyces carsonii*; *Pichia* sp., especially *Pichia anomala* and *Pichia kluyveri*; and *Candida* sp., especially *Candida krusei*. The ITS DNA sequences, as well as primers of interest, are set forth in SEQUENCE ID NOS: 1-100. The sequences are useful in PCR-based identification of fermentation-related microorganisms.

Methods for use of the primer sequences of the invention in PCR analysis are well known in the art. For example, see U.S. Patent Nos. 4,683,195; 4,683,202 and 5,585,238, the contents of all of which are hereby incorporated by reference.

The primer sequences of the invention can also be used as molecular probes to detect the presence of target DNA. The  $T_m$  for the primers ranges from about 48-58° C at 50 mM salt. The hybridization temperature is approximately 5-10° C below the melting temperature. Thus, the primers are hybridized to target DNA typically at a temperature ranging from about 43-55° C. Final wash conditions generally range from about 45-55° C at about 36 mM salt concentration. Specific hybridization as used herein means the use of a final high stringency wash in about 0.2X SSPE (salt concentration of about 36 mM) at a temperature appropriate for the particular primer. 1X SSPE contains 10 mM  $\text{NaH}_2\text{PO}_4$ , 180 mM NaCl, and 1 mM EDTA, at pH 7.4.

The ITS DNA sequences of the present invention can be cloned from fermentation-related microorganisms by methods known in the art. In general, the methods for the isolation of DNA from microorganism isolates are known. See, Raeder & Broda (1985) *Letters in Applied Microbiology* 2:17-20; Lee et al. (1990) *Fungal Genetics Newsletter* 35:23-24; and Lee and Taylor (1990) In: *PCR Protocols: A Guide to Methods and Applications*, Innes et al. (Eds.); pages 282-287; the contents of all of which are hereby incorporated by reference.

Alternatively, the ITS regions of interest can be identified by PCR amplification. Primers to amplify the entire ITS region can be synthesized according to White et al. (1990; In *PCR Protocols*; Eds.: Innes et al., pages 315-322, the contents of which are hereby incorporated by reference).

The ITS sequences were determined and the sequences were compared to locate divergences which might be useful to test in PCR to distinguish the different fermentation-related microorganisms. The sequences of the ITS regions which were determined are shown as Sequence ID NOS: 1 to 12. The DNA sequences for the ITS1 and ITS2 regions are shown as Sequence ID NOS: 13 to 36. From the identification of divergences, numerous primers were synthesized and tested in PCR-amplification. Purified microorganism

DNA and DNA isolated from fermentation cultures and colonies were used as templates for PCR-amplification. Thus, pairs of diagnostic primers were identified, *i.e.*, those which identified one particular fermentation-related microorganism species. Preferred primer combinations are able to distinguish  
5 between the different microorganisms in, for example, fermentation cultures. Primer sequences are set forth in Sequence ID NOS: 65 to 98, with flanking sequences depicted in Sequence ID NOS: 37 to 64. Thus, while oligonucleotide primers selected from among Sequence ID NOS: 65 to 98 are preferred, primers may also be used which contain at least 10 contiguous  
10 nucleotide bases from one of SEQ ID NOS: 65 to 98. Additionally, primers may be used which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64, and primers of 10 nucleotide bases or longer which contain at least 5 contiguous nucleotide bases  
15 from one of SEQ ID NOS: 65 to 98 contiguous with from 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64.

The present invention provides numerous diagnostic primer combinations. The primers of the invention are designed based on sequence differences among the microorganism ITS regions. A minimum of one base  
20 pair difference between sequences can permit design of a discriminatory primer. In general, primers should have a theoretical melting temperature between about 55 °C to about 65 °C to achieve good sensitivity, and should be void of significant secondary structure and 3' overlaps between primer combinations. Primers are generally at least about 10 nucleotide bases, more  
25 preferably at least about 15 to about 20 nucleotide bases.

The oligonucleotide primers of the present invention are particularly useful in detecting microorganisms involved in fermentations, in particular, microorganisms selected from among *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomycodes ludwigii*, *Dekkera bruxellensis*,

*Dekkera intermedia*, *Botrytis cinerea*, *Penicillium crustosum*, *Penicillium expansum*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*, *Pichia anomala*, *Pichia kluyveri*, and *Candida krusei*. However, the primers of the present invention can also be used to detect the presence of the foregoing  
5 microorganisms from any source.

The present invention also relates to the preparation of "kits" containing elements for detecting fermentation-related microorganisms. Such a kit may comprise a carrier to receive therein one or more containers, such as tubes or vials. Unlabeled or detectably labeled oligonucleotide primers may be  
10 contained in one or more of the containers. The oligonucleotide primers may be present in lyophilized form, or in an appropriate buffer. One or more enzymes or reagents for use in PCR reactions may be contained in one or more of the containers. The enzymes or reagents may be present alone or in admixture, and in lyophilized form or in appropriate buffers. The kit may also  
15 contain any other component necessary for carrying out the present invention, such as buffers, extraction agents, enzymes, pipettes, plates, nucleic acids, nucleoside triphosphates, filter paper, gel materials, transfer materials, and autoradiography supplies.

The examples below illustrate typical experimental protocols which can  
20 be used in the isolation of ITS sequences, the selection of suitable primer sequences, the testing of primers for selective and diagnostic efficacy, and the use of such primers to detect the presence of a fermentation-related microorganism. Such examples are provided by way of illustration and not by way of limitation.

## Examples

### Example 1

#### Culture of Yeast and Fungal Isolates

5        Viable isolates of *Botrytis cinerea*, *Penicillium crustosum*, and  
      *Penicillium expansum* were obtained from the American Type Culture  
Collection (ATCC). Fungi were grown in 40 ml of Malt Yeast Extract Broth  
in 250 ml flasks inoculated with mycelial fragments from two-week-old  
cultures grown on Malt Yeast Extract Agar (MYEA). Liquid cultures were  
incubated at room temperature for 14 days without shaking. Malt Yeast  
10    Extract Agar plates were inoculated with mycelia and grown for 2 weeks.  
      Viable isolates of *Saccharomyces cerevisiae*, *Saccharomyces bayanus*,  
*Saccharomycodes ludwigii*, *Dekkera bruxellensis*, *Dekkera intermedia*, *Dekkera*  
*anomala*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*, *Pichia*  
*anomala*, *Pichia kluyveri*, and *Candida krusei* were obtained from the ATCC or  
15    from the Gallo Sonoma Winery (see Table 1). Yeasts were grown on any of  
several media of choice.

### Example 2

#### Amplification and Sequencing of the Internal Transcribed Spacer (ITS) Regions

      The internal transcribed spacer region was amplified from the different  
20    isolates directly from the fungal mycelium or the yeast colony using ITS5  
(5'-GGAAGTAAAAGTCGTAACAAGG-3'; SEQ ID NO: 99) and ITS4  
(5'-TCCTCCGCTTATTGATATGC-3'; SEQ ID NO: 100). A sterile pipette  
tip was used to scrape a small amount of mycelia or colony off of the plate and  
deposited into a 250- $\mu$ l microcentrifuge tube containing 5  $\mu$ l each of GeneAmp®  
25    10X PCR Buffer II and MgCl<sub>2</sub> solution (PE Applied Biosystems, Foster City,  
CA; part no. N808-0161), 0.2 mM each of dATP, dCTP, dGTP, and dTTP



(GeneAmp® dNTPs; PE Applied Biosystems, Foster City, CA; part no. N808-0007), approximately 25 pmole/ $\mu$ l each of ITS5 and ITS4, and 2.5 Units AmpliTaq® DNA polymerase (PE Applied Biosystems; part no. N808-0161). Reactions were run for 35 cycles of 30 s at 94 °C, 40 s at 58 °C, and 2 min at 72 °C, followed by a final elongation step at 72 °C for 10 min, on a Perkin Elmer GeneAmp® PCR System 9600 (PE Applied Biosystems). PCR products were purified using QIAquick® PCR Purification Kits (Qiagen Inc., Santa Clarita, CA) to remove any excess primers, nucleotides, and polymerases. Five microliters of the purified PCR products were run on a 1.2% agarose gel with 5  $\mu$ l of pGEM®-3Zf(+) double-stranded DNA Control Template (0.2 g/L, PE Applied Biosystems) to approximate concentrations. All products were sequenced using the primers ITS5 and ITS4 (see sequences above; White et al., 1990; In: *PCR Protocols*; Eds.: Innes et al. pp. 315-322). Sequencing was performed on an PE Applied Biosystems 377 Automated DNA Sequencer® using ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kits® (PE Applied Biosystems; part no. 402079). Cycle sequencing products were run over Centri-Sep® spin columns (Princeton Separations, Inc., Adelphia, NJ) to remove excess primers, dye-labeled terminators, nucleotides, and polymerases before being run on the automated sequencer.

20

### Example 3

#### Selection of Species-Specific Primers

The ITS sequences of the *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomycodes ludwigii*, *Dekkera bruxellensis*, *Dekkera intermedia*, *Dekkera anomala*, *Botrytis cinerea*, *Penicillium crustosum*, *Penicillium expansum*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*, *Pichia anomala*, *Pichia kluyveri*, and *Candida krusei* isolates were aligned and

25

primers were designed using Oligo 5.0 (National Biosciences, Inc., Plymouth, MN) in regions of maximum sequence difference between the target species.

#### Example 4

##### Primer Synthesis

5        Primers were synthesized on an Applied Biosystems 394 DNA/RNA Synthesizer® using phosphoramidite chemistry.

#### Example 5

##### Verification of Primer Specificity to Target Species

10        Different annealing temperatures were tested to determine the optimal temperature for PCR for individual primers. In cases with multiple species-specific primers, different primer combinations were used to determine the best primer combination and annealing temperature to amplify a single species-specific DNA fragment. Species-specific amplification products were produced from primers designed from the ITS region between the 18S and 28S ribosomal  
15        DNA subunits of each fungal strain of interest.

#### Example 6

##### Utilization of ITS sequences as diagnostic probes to hybridize with target DNA

1. Put chosen concentration of DNA sample in 100 ul of TE, pH 7.0.
2. Add 0.1 volume [10  $\mu$ l] of 3.0 M NaOH, vortex to mix and incubate  
20        at  
65° C for 20 min to destroy the RNA and denature the DNA.
3. Spin down condensation. Allow samples to cool to room temp.  
Neutralize by adding 1.0 volume [110  $\mu$ l] of 2M ammonium acetate, pH 7.0,

vortex to mix. Spin down to remove solution off of cap. Refrigerate until slot blot apparatus is ready.

4. Apply to slot-blot apparatus according to manufacturers protocol; about 220  $\mu$ l to slot blot.

5           5. Label ITS sequence probe according to kit manufacturer's recommendation.

6. Prehybridize blots in 1.0% BSA; 1mM EDTA, 0.5 M  $\text{NaHPO}_4$ , pH 7.2, 7.0% sodium dodecyl sulfate for a minimum of 2 hr prior to adding the probe, and then hybridized for 16 hr at 45° C. Initial washes consist of two 30-  
10 min washes in 1X SSPE/0.1% SDS at 50° C. Transfer blots to a plastic tray and wash in 1X SSPE for 1 hr, at 50° C with shaking. The final wash should consist of 15 min at 50° C in 0.2X SSPE.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that  
15 various changes and modifications can be made therein without departing from the spirit and scope thereof.

Table 1  
Sources of test isolates

	Species name	ID number	Source
	<i>Saccharomyces cerevisiae</i>	GS061	Gallo of Sonoma <sup>1</sup>
5	<i>Saccharomyces cerevisiae</i>	GS117	Gallo of Sonoma
	<i>Saccharomyces cerevisiae</i>	4127	ATCC <sup>2</sup>
	<i>Saccharomyces bayanus</i>	13056	ATCC
	<i>Saccharomyces ludwigii</i>	34085	ATCC
	<i>Saccharomyces ludwigii</i>	44299	ATCC
10	<i>Dekkera bruxellensis</i>	Y153	Gallo of Sonoma
	<i>Dekkera bruxellensis</i>	Y207	Gallo of Sonoma
	<i>Dekkera bruxellensis</i>	10560	ATCC
	<i>Dekkera intermedia</i>	34448	ATCC
	<i>Dekkera anomala</i>	10559	ATCC
15	<i>Botrytis cinerea</i>	20599	ATCC
	<i>Penicillium crustosum</i>	58616	ATCC
	<i>Penicillium expansum</i>	28885	ATCC
	<i>Hanseniaspora guilliermondii</i>	GS014	Gallo of Sonoma
	<i>Hanseniaspora guilliermondii</i>	GS057	Gallo of Sonoma
20	<i>Hanseniaspora guilliermondii</i>	66166	ATCC
	<i>Debaryomyces carsonii</i>	Y443	Gallo of Sonoma
	<i>Debaryomyces carsonii</i>	Y448	Gallo of Sonoma
	<i>Debaryomyces carsonii</i>	24214	ATCC
	<i>Pichia anomala</i>	GS085	Gallo of Sonoma
25	<i>Pichia anomala</i>	34080	ATCC
	<i>Pichia kluyveri</i>	GS070	Gallo of Sonoma
	<i>Pichia kluyveri</i>	64303	ATCC
	<i>Candida krusei</i>	GS076	Gallo of Sonoma
	<i>Candida krusei</i>	GS096	Gallo of Sonoma
30	<i>Candida krusei</i>	GS108	Gallo of Sonoma

<sup>1</sup>Gallo of Sonoma Winery, Healdsburg, CA, USA

<sup>2</sup>American Type Culture Collection, Rockville, MD, USA

Table 2

	Target Organism	Primer Name	Primer Sequence
	<i>Saccharomyces</i> sp.	SXU99	5'-CTTTTACTGGGCAAGAAGAC-3' (SEQ ID NO: 65)
	<i>Saccharomyces</i> sp.	SXL658	5'-AAGCACGCAGAGAAACC-3' (SEQ ID NO: 66)
5	<i>Saccharomyces</i> sp.	SXU102	5'-TTACTGGGCAAGAAGACAAG-3' (SEQ ID NO: 67)
	<i>Saccharomyces</i> sp.	SXL661	5'-CTCAAGCACGCAGAGAA-3' (SEQ ID NO: 68)
	<i>Saccharomycodes ludwigii</i>	SLU85	5'-GACTTTTCTTGGGGAGAG-3' (SEQ ID NO: 69)
10	<i>Saccharomycodes ludwigii</i>	SLL635	5'-TCACTAGTTGGGATAAACCT-3' (SEQ ID NO: 70)
	<i>Saccharomycodes ludwigii</i>	SLU88	5'-TTTTCTTGGGGAGAGG-3' (SEQ ID NO: 71)
	<i>Saccharomycodes ludwigii</i>	SLL636	5'-ATCACTAGTTGGGATAAACCT-3' (SEQ ID NO: 72)
15	<i>Saccharomycodes ludwigii</i>	SLU136	5'-CGGCTAGTAGTTGATGATT-3' (SEQ ID NO: 73)
	<i>Saccharomycodes ludwigii</i>	SLL634	5'-CACTAGTTGGGATAAACCTA-3' (SEQ ID NO: 74)
	<i>Dekkera</i> sp.	BRU53A	5'-ATTACAGGATGCTGGGC-3' (SEQ ID NO: 75)
20	<i>Dekkera</i> sp.	BRU53B	5'-ATTACAGGATGCTGGG-3' (SEQ ID NO: 76)
	<i>Dekkera</i> sp.	BRU77	5'-CGTGCAGACACGTGGAT-3' (SEQ ID NO: 77)
	<i>Dekkera</i> sp.	BRL339	5'-CTTTGAAGAAAACACCCTC-3' (SEQ ID NO: 78)
	<i>Dekkera</i> sp.	BRL367	5'-AATCATTATCCCCTCACTC-3' (SEQ ID NO: 79)

	Target Organism	Primer Name	Primer Sequence
	<i>Dekkera</i> sp.	BRL390	5'-TAATGAACGGCCGAAAC-3' (SEQ ID NO: 80)
	<i>Botrytis cinerea</i>	BCU136	5'-TTGTATGCTCGCCAGAG-3' (SEQ ID NO: 81)
	<i>Botrytis cinerea</i>	BCL393	5'-GCCTGCCATTACTGACA-3' (SEQ ID NO: 82)
	<i>Botrytis cinerea</i>	BCU142	5'-GCTCGCCAGAGAATACC-3' (SEQ ID NO: 83)
5	<i>Penicillium</i> sp.	PXU87	5'-ACCCGTCTTTATTTTACCTT-3' (SEQ ID NO: 84)
	<i>Penicillium</i> sp.	PXL495	5'-CTACAGAGCGGGTGACA-3' (SEQ ID NO: 85)
	<i>Penicillium</i> sp.	PXU86	5'-CACCCGTGTTTATTTTACCT-3' (SEQ ID NO: 86)
	<i>Penicillium</i> sp.	PXL482	5'-CAAAGCCCCATACGC-3' (SEQ ID NO: 87)
	<i>Penicillium</i> sp.	PXL491	5'-AGCGGGTGACAAAGC-3' (SEQ ID NO: 88)
10	<i>Hanseniaspora guilliermondii</i>	HGU193	5'-CAACGTTTACACACATTGG-3' (SEQ ID NO: 89)
	<i>Hanseniaspora guilliermondii</i>	HGU231	5'-AATTCTTTCTGCTTTGAATCG-3' (SEQ ID NO: 90)
15	<i>Hanseniaspora guilliermondii</i>	HGL601	5'-CAGCGTCTCCAAAGAAGCTAA-3' (SEQ ID NO: 91)
	<i>Debaryomyces carsonii</i>	DXL447	5'-GCAAACGCCTAGTTCGACTAA-3' (SEQ ID NO: 92)
	<i>Debaryomyces carsonii</i>	DXL526	5'-ATTCAACGAGTTGGATAAACC-3' (SEQ ID NO: 93)
20	<i>Pichia anomala</i>	PAU133	5'-GGCTTACTGCCCAAAGGTC-3' (SEQ ID NO: 94)
	<i>Pichia anomala</i>	PAL451	5'-TGCTTATTAGTACACTCTTGC-3' (SEQ ID NO: 95)

Target Organism	Primer Name	Primer Sequence
<i>Pichia kluyveri</i>	PKL356	5'-GTTTAGTTCACTTCGTCCACG-3' (SEQ ID NO: 96)
<i>Candida krusei</i>	CKU104	5'-CCTAAAATGTGGAATATAGCA-3' (SEQ ID NO: 97)
<i>Candida krusei</i>	CKL354	5'-ACGCTCTTTACACGTCGTC-3' (SEQ ID NO: 98)

Table 3

## ITS-derived Diagnostic PCR Primers

	Target Organism	5' Primer	3' Primer	Approximate Size of PCR Product (bp)
5	<i>Saccharomyces sp.</i>	SXU99	ITS4	750
	<i>Saccharomyces sp.</i>	SXU102	ITS4	750
	<i>Saccharomyces sp.</i>	ITS5	SXL658	695
	<i>Saccharomyces sp.</i>	ITS5	SXL661	695
	<i>Saccharomyces sp.</i>	SXU99	SXL658	560
10	<i>Saccharomyces sp.</i>	SXU99	SXL661	560
	<i>Saccharomyces sp.</i>	SXU102	SXL658	560
	<i>Saccharomyces sp.</i>	SXU102	SXL661	560
	<i>Saccharomycodes ludwigii</i>	SLU85	ITS4	660
	<i>Saccharomycodes ludwigii</i>	SLU88	ITS4	660
15	<i>Saccharomycodes ludwigii</i>	SLU136	ITS4	610
	<i>Saccharomycodes ludwigii</i>	ITS5	SLL635	670
	<i>Saccharomycodes ludwigii</i>	ITS5	SLL636	670
	<i>Saccharomycodes ludwigii</i>	ITS5	SLL634	670
	<i>Saccharomycodes ludwigii</i>	SLU85	SLL635	565
20	<i>Saccharomycodes ludwigii</i>	SLU85	SLL636	565
	<i>Saccharomycodes ludwigii</i>	SLU85	SLL634	565
	<i>Saccharomycodes ludwigii</i>	SLU88	SLL635	565
	<i>Saccharomycodes ludwigii</i>	SLU88	SLL636	565
	<i>Saccharomycodes ludwigii</i>	SLU88	SLL634	565
25	<i>Saccharomycodes ludwigii</i>	SLU136	SLL635	500
	<i>Saccharomycodes ludwigii</i>	SLU136	SLL636	500
	<i>Saccharomycodes ludwigii</i>	SLU136	SLL634	500
	<i>Dekkera sp.</i>	BRU53A	ITS4	450
	<i>Dekkera sp.</i>	BRU53B	ITS4	450
30	<i>Dekkera sp.</i>	BRU77	ITS4	450
	<i>Dekkera sp.</i>	ITS5	BRL339	375
	<i>Dekkera bruxellensis</i>	ITS5	BRL367	400
	<i>Dekkera sp.</i>	ITS5	BRL390	425
	<i>Dekkera sp.</i>	BRU53A	BRL339	290
35	<i>Dekkera bruxellensis</i>	BRU53A	BRL367	320
	<i>Dekkera sp.</i>	BRU53A	BRL390	340



	<i>Dekkera sp.</i>	BRU53B	BRL339	290
	<i>Dekkera bruxellensis</i>	BRU53B	BRL367	320
	<i>Dekkera sp.</i>	BRU53B	BRL390	340
	<i>Dekkera sp.</i>	BRU77	BRL339	260
5	<i>Dekkera bruxellensis</i>	BRU77	BRL367	290
	<i>Dekkera sp.</i>	BRU77	BRL390	310
	<i>Botrytis cinerea</i>	BCU136	ITS4	350
	<i>Botrytis cinerea</i>	BCU142	ITS4	345
	<i>Botrytis cinerea</i>	ITS5	BCL393	420
10	<i>Botrytis cinerea</i>	BCU136	BCL393	270
	<i>Botrytis cinerea</i>	BCU142	BCL393	265
	<i>Penicillium sp.</i>	PXU87	ITS4	410
	<i>Penicillium sp.</i>	PXU86	ITS4	410
	<i>Penicillium sp.</i>	ITS5	PXL495	530
15	<i>Penicillium sp.</i>	ITS5	PXL482	520
	<i>Penicillium sp.</i>	ITS5	PXL491	530
	<i>Penicillium sp.</i>	PXU87	PXL495	405
	<i>Penicillium sp.</i>	PXU87	PXL482	395
	<i>Penicillium sp.</i>	PXU87	PXL491	405
20	<i>Hanseniaspora guilliermondii</i>	HGU193	ITS4	530
	<i>Hanseniaspora guilliermondii</i>	HGU231	ITS4	490
	<i>Hanseniaspora guilliermondii</i>	ITS5	HGL601	630
25	<i>Hanseniaspora guilliermondii</i>	HGU193	HGL601	420
	<i>Hanseniaspora guilliermondii</i>	HGU231	HGL601	380
30	<i>Debaryomyces carsonii</i>	ITS5	DXL447	480
	<i>Debaryomyces carsonii</i>	ITS5	DXL526	560
	<i>Pichia anomala</i>	PAU133	ITS4	505
	<i>Pichia anomala</i>	ITS5	PAL451	480
	<i>Pichia anomala</i>	PAU133	PAL451	320
35	<i>Pichia kluyveri</i>	ITS5	PKL356	390
	<i>Candida krusei</i>	CKU104	ITS4	440
	<i>Candida krusei</i>	ITS5	CKL354	385

We claim:

1. An isolated double stranded nucleic acid selected from the group consisting of SEQ ID NOS: 13 to 36.
2. An isolated nucleic acid which specifically hybridizes with the  
5 nucleic acid of claim 1.
3. An oligonucleotide sequence for identification of a fermentation-related microorganism, wherein said sequence is selected from the group consisting of SEQ ID NOS: 37 to 64.
4. An oligonucleotide primer which is a fragment of the sequences  
10 according to claim 3, and which specifically hybridizes to the ITS1 or ITS2 of *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomycodes ludwigii*, *Dekkera bruxellensis*, *Dekkera intermedia*, *Botrytis cinerea*, *Penicillium crustosum*, *Penicillium expansum*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*, *Pichia anomala*, *Pichia kluyveri* or *Candida krusei*.
- 15 5. An oligonucleotide primer for identification of a fermentation-related microorganism, wherein said primer is selected from the group consisting of primers which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98, primers which contain at least 10 contiguous nucleotide  
20 bases from one of SEQ ID NOS: 65 to 98 contiguous with 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64, and primers of 10 nucleotide bases or longer which contain at least 5 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with from 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64.

6. A pair of oligonucleotide primers for use in the amplification-based detection of an internal transcribed spacer sequence of a fermentation-related microorganism, wherein the primers are selected from the group consisting of primers which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98, primers which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64, and primers of 10 bases or longer which contain at least 5 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with from 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64.

7. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 65 and SEQ ID NO: 66.

8. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 67 and SEQ ID NO: 68.

9. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 69 and SEQ ID NO: 70.

10. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 71 and SEQ ID NO: 72.

11. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 73 and SEQ ID NO: 74.

12. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 75 and SEQ ID NO: 78.

13. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 76 and SEQ ID NO: 79.

14. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 77 and SEQ ID NO: 80.

5           15. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 81 and SEQ ID NO: 82.

16. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 85 and SEQ ID NO: 83.

10           17. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 84 and SEQ ID NO: 85.

18. The pair oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 86 and SEQ ID NO: 87.

19.     The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 86 and SEQ ID NO: 88.

15           20. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 89 and SEQ ID NO: 91.

21. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 90 and SEQ ID NO: 91.

20           22. The pair oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 94 and SEQ ID NO: 95.

23. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 97 and SEQ ID NO: 98.

24. A method for detection of a fermentation-related microorganism comprising:

- 5 (a) obtaining DNA from a fungal culture or colony isolated from a fermentation, or from an organism present in a fermentation beverage;
- (b) amplifying a part of the internal transcribed spacer sequence of said fermentation-related microorganism using said DNA as a  
10 template in a polymerase chain reaction with a pair of oligonucleotide primers according to claim 6; and
- (c) visualizing said amplified part of the internal transcribed spacer sequence to determine whether said fermentation-related microorganism is present.

15 25. The method according to claim 24, wherein said fermentation-related microorganism is selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomycodes ludwigii*, *Dekkera bruxellensis*, *Dekkera intermedia*, *Botrytis cinerea*, *Penicillium crustosum*, *Penicillium expansum*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*,  
20 *Pichia anomala*, *Pichia kluyveri*, and *Candida krusei*.

26. The method according to claim 24, wherein said fermentation culture or fermentation beverage is a wine fermentation culture or wine fermentation beverage.

27. The method according to claim 24, wherein the pair of  
25 oligonucleotide primers comprises SEQ ID NO: 65 and SEQ ID NO: 66.

28. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 67 and SEQ ID NO: 68.

29. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 69 and SEQ ID NO: 70.

5 30. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 71 and SEQ ID NO: 72.

31. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 73 and SEQ ID NO: 74.

10 32. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 75 and SEQ ID NO: 78.

33. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 76 and SEQ ID NO: 79.

34. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 77 and SEQ ID NO: 80.

15 35. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 81 and SEQ ID NO: 82.

36. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 82 and SEQ ID NO: 83.

20 37. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 84 and SEQ ID NO: 85.

38. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 86 and SEQ ID NO: 87.

39. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 86 and SEQ ID NO: 88.

5           40. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 89 and SEQ ID NO: 91.

41. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 90 and SEQ ID NO: 91.

10           42. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 94 and SEQ ID NO: 95.

43. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 97 and SEQ ID NO: 98.

15           44. A kit comprising a carrier to receive therein one or more containers, at least one of said containers comprising an oligonucleotide primer according to claim 5.

45. A kit comprising a carrier to receive therein one or more containers, at least one of said containers comprising a pair of oligonucleotide primers according to claim 6.

Sequence Listing

SEQ ID NO: 1      DNA sequence for the internal transcribed spacer of  
*Saccharomyces cerevisiae* and *Saccharomyces bayanus*.

5'      AAGAAATTTAATAATTTTGAAAATGGATTTTTTTTGT  
5      GGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAGACAA  
GAGATGGAGAGTCCACCCGGGCCTGCGCTTAAGTGCGC  
GGTCTTGCTAGGCTTGTAAGTTTCTTTCTTGCTATTCCA  
AACGGTGAGAGATTTCTGTGCTTTTGTATAGGACAATT  
AAAACCGTTTCAATACAACACACTGTGGAGTTTTCATAT  
10      CTTTGCAACTTTTCTTTGGGCATTTCGAGCAATCGGGGC  
CCAGAGGTAACAAACACAAACAATTTTATCTATTCATTA  
AATTTTTGTCAAAAACAAGAATTTTCGTAACCTGGAAATT  
TAAAAATATTAAAAACTTTCAACAACGGATCTCTTGGTT  
CTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTA  
15      ATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGA  
ACGCACATTGCGCCCTTGGTATTCCAGGGGGCATGCCT  
GTTTGAGCGTCATTTCTTCTCAAACATTCTGTTTGGTA  
GTGAGTGATACTCTTTGGAGTTAACTTGAAATTGCTGGC  
CTTTTCATTGGATGTTTTTTTTTCCAAAGAGAGGTTTCTC  
20      TCGTGCTTGAGGTATAATGCAAGTACGGTCGTTTTAGG  
TTTTACCAACTGCGGCTAATCTTTTTTTATACTGAGCGT  
ATTGGAACGTTATCGATAAGAAGAGAGCGTCTAGGCGA  
ACAATGTTCTTAAAGT 3'

25      SEQ ID NO: 2      DNA sequence for the internal transcribed spacer of  
*Saccharomyces ludwigii*.

5'      AAGAAAAAACTGTTTATAAACAGACGGTAGACTTTTC  
TTGGGGAGAGGTTGTTGATTGCTTTGGCCTGCGCTTAAC  
TGCGCGGCTAGTAGTTGATGATTTTGTTATTATCCGAGA  
CGAAGGAAACGTCTGATTTTAAAAACATTATACACTTTG  
30      GAGTACTTTTTTAAATGTATTTCTTCTTGGACGAGCAA  
TTGTTCAAGGGTCAATAAACACAAACAATTTTTTTTTTA  
TTTAAATTTAAATAATTCAAAATATATCATTCTTTTA  
TTAGGAATATAAAAAATTTTAAACTTTCAACAACGGATC  
TCTTGGTTCTCGCATCGATGAAGAACGTAGCGAATTGCG  
35      ATAAGTAATGTGAATTGCAGATTTTCGTGAATCATTGAA  
TTTTTGAACGCACATTGCGCCCTTGGTATTCCAAAGGG  
CATGCCTGTTTGAGCGTCATTTCTTCTCAAAGAGTTT  
TTTTATTCTTTTGGTTGTGAGTGATACTCTTCTTTTAC



AGGGAAGGGGTAACTTGAAATTGTTGCCTAGCAAAGA  
AGAATTTTGATTGAAATTTCTTGTTTATTACTATTAGGT  
TTATCCCAACTAGTGATTATTGAGAGTTTTTATTACAGA  
GTCATTTCACTTGCTATAATACTATTCTATAAGT 3'

5 SEQ ID NO: 3 DNA sequence for the internal transcribed spacer of  
*Dekkera bruxellensis*.

5' CAGGATGCTGGGCGCAAGCCCGTGCAGACACGTGGATA  
AGCAAGGATAAAAATACATTAAATTTATTTAGTTTAGTC  
AAGAAAGAATTTTAACTTTCAACAATGGATCTCTTGG  
10 TTCTCGCGTCGATGAAGAGCGCAGCGGAATTGCGATAC  
TTAATGTGAATTGCAGATTTTCGTGAATCATCGAGTTCT  
TGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCAT  
GCCTGTTTGAGCGTCATTTCTTCTCACTATTTAGTGGT  
TATGAGATTACACGAGGGTGTTTCTTCAAAGGAAAGA  
15 GGGGAGAGTGAGGGGATAATGATTAAAGGTTTCGGCCG  
TTCATTATTTTTTCTTCTCCCCCAGTTATCAAGT 3'

SEQ ID NO: 4 DNA sequence for the internal transcribed spacer of  
*Dekkera intermedia*.

5' CAGGATGCTGGGCGCAAGCCCGTGCAGACACGTGGATA  
20 AGTAAGGATAAAAATACATTAAATTTATTTAGTTTAGT  
CAAGAAAGAATTTTAACTTTCAACAATGGATCTCTTG  
GTTCTCGCGTCGATGAAGAGCGCAGCGAATTGCGATAC  
TTAATGTGAATTGCAGATTTTCGTGAATCATCGAGTTCT  
TGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCAT  
25 GCCTGTTTGAGCGTCATTTCTTCTCACTATTTAGTGGT  
TATGAGATTACACGAGGGTGTTTCTTCAAAGGAAAGA  
GGGGAGAGTGAGGGGATAATGATTAAAGGTTTCGGCCG  
TTCATTATTTTTTCTTCTCCCCCAGTTATCAAGT 3'

30 SEQ ID NO: 5 DNA sequence for the internal transcribed spacer of  
*Botrytis cinerea*.

5' CAGAGTTCATGCCCCGAAAGGGTAGACCTCCCACCCTTG  
TGTATTATTACTTTGTTGCTTTGGCGAGCTGCTTTCGGG  
CCTTGTATGCTCGCCAGAGAATAACCAAACTCTTTTAT  
TAATGTCGTCTGAGTACTATATAATAGTTAAAACTTCA

5 ACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC  
AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAG  
TGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGG  
TATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTTCAACC  
CTCAAGCTTAGCTTGGTATTGAGTCTATGTCAGTAATGG  
CAGGCTCTAAAATCAGTGGCGGGCGCCGCTGGGTCTCTGA  
ACGTAGTAATATCTCTCGTTACAGGTTCTCGGTGTGCTT  
CTGCCAAAACCCAAATTTTCTATGG 3'

10 SEQ ID NO: 6 DNA sequence for the internal transcribed spacer of  
*Penicillium crustosum*.

5' CCGAGTGAGGGGCCCTCTGGGTCCAACCTCCCACCCGTG  
TTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAAC  
GGCCGCCGGGGGGCTTACGCCCCCGGGCCCGCGCCCGC  
15 CGAAGACACCCTCGAACTCTGTCTGAAGATTGAAGTCT  
GAGTGAAAATATAAATTATTTAAACTTTCAACAACGG  
ATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAA  
TGCGATACGTAATGTGAATTGCAAATTCAGTGAATCATC  
GAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGG  
GGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCC  
20 GGCTTGTGTGTTGGGGCCCCGTCCCCCGATCTCCGGGGGA  
CGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCTT  
CGAGCGTATGGGGCTTTGTACCCCGCTCTGTAGGCCCGG  
CCGGCGCTTGCCGATCAACCCAAATTTTATCCAGG 3'

25 SEQ ID NO: 7 DNA sequence for the internal transcribed spacer of  
*Penicillium expansum*.

5' CCGAGTGAGGGGCCCTTTGGGTCCAACCTCCCACCCGTGT  
TTATTTACCTCGTTGCTTCGGCGGGCCCGCCTTAAC  
CCGCCGGGGGGCTCACGCCCCCGGGCCCGCGCCCGCCG  
30 AAGACACCCCCGAACCTCTGCCTGAAGATTGTCGTCTGA  
GTGAAAATATAAATTATTTAAACTTTCAACAACGGATC  
TCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGC  
GATACGTAATGTGAATTGCAAATTCAGTGAATCATCGA  
GTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGG  
GCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGG  
35 CTTGTGTGTTGGGGCCCCGTCCGATTCGGGGGGACGG  
GCCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCTCTGA  
GCGTATGGGGCTTTGTACCCCGCTCTGTAGGCCCGGCCG  
GCGCTTGCCGATCAACCCAAATTTTATCCAGGTTGACC

TCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATAT  
CAATAAGCGGAGGAAA 3'

SEQ ID NO: 8 DNA sequence for the internal transcribed spacer of  
*Hanseniaspora guilliermondii*.

5 5' GATTGAATTATCATTGTTGCTCGAGTTCTAGTTTTAGAT  
CTTTTACAATAATGTGTATCTTTATTGAAGATGTGCGCT  
TAATTGCGCTGCTTTTTTAAAGTGTCGCAGTAGAAGTAA  
TCTTGCTTGAATCTCAGTCAACGTTTACACACATTGGAG  
TTTTTTTACTTTAATTTAATTCTTTCTGCTTTGAATCGAA  
10 AGGTTCAAGGCAAAAAACAAACACAAACAATTTTATTT  
TATTATAATTTTTTAAACTAAACCAAAATTCCTAACGGA  
AATTTTAAAATAATTTAAAACCTTCAACAACGGATCTCT  
TGGTTCTCGCATCGATGAAGAACGTAGCGAATTGCGAT  
AAKTAATGTGAATTGCAGATACTCGTGAATCATTGAATT  
15 TTTGAACGCACATTGCGCCCTTGAGCATTCTCAAGGGCA  
TGCCTGTTTGAGCGTCATTTCTTCTCAAAGATAATTT  
TTTATTTTTTGGTTGTGGGCGATACTCAGGGTTAGCTTG  
AAATTGAAGATTGTTTCAATCTTTTTTAATTCAACACTT  
AGCTTCTTTGGAGACGCTGTTCTCGCTGTGATGTATTTA  
20 TGAATTTATTTCGTTTTACTTTACAAGGGAAATGGTAATG  
TACCTTAGGCAAAGGGTTGCTTTTAATATTCATCAAGT  
3'

SEQ ID NO: 9 DNA sequence for the internal transcribed spacer of  
*Debaryomyces carsonii*.

25 5' CAGTATTCTTTTTTGCCAGCGCTTAACTGCGCGGCGAAAT  
AAACCTTACACACAATGTTTTTTGTTATTACAGGAACCTT  
TTGCTTTGGCTTGTCTCTAGAAATAGAGTTGGGCCAAAG  
GTTTAAACTAACTTCAATTTATTTGAACTATTTTTCTTAT  
TGAAATGTCACTTTGTTGATTAAATTCAAAAAATCTTCA  
30 AAACCTTCAACAACGGATCTCTTGGTTCTCGCATCGATG  
AAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCA  
GATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCG  
CCCTTTGGTATTCCAAAGGGCATGCCTGTTTGAGCGTCA  
TTTCTCTCTCAAACCTTAGGGTTTGGTATTGAGTGATAC  
35 TCTTAGTCGAACCTAGGCGTTTGCTTGAAATGTATCGGCA  
TGAGTGGTACTAGATTAGTGCTTCAGATTTTTCAATGTA  
TTAGGTTTATCCAACCTCGTTGAATAGTCTGATGGCAAGT

GTTTAGTAACTATGGCTCGGCCTAACAACAACAAACAA  
GT 3'

SEQ ID NO: 10 DNA sequence for the internal transcribed spacer of  
*Pichia anomala*.

5 5' TAGTATTCTATTGCCAGCGCTTAATTGCGCGGCGATAAA  
CCTTACACACATTGTCTAGTTTTTTTTGAACTTTGCTTTGG  
GTGGTGAGCCTGGCTTACTGCCCAAAGGTCTAAACACA  
TTTTTTTAATGTTAAACCTTTAACCAATAGTCATGAAA  
ATTTTTAACAAAAATTAAAATCTTCAAAACTTTCAACAA  
10 CGGATCTCTTGTTCTCGCAACGATGAAGAACGCAGCG  
AAATGCGATACGTATTGTGAATTGCAGATTTTCGTGAAT  
CATCGAATCTTTGAACGCACATTGCACCCTCTGGTATTC  
CAGAGGGTATGCCTGTTTGAGCGTCATTTCTCTCTCAA  
CCTTCGGGTTTGGTATTGAGTGATACTCTGTCAAGGGTT  
15 AACTTGAAATATTGACTTAGCAAGAGTGTAATAAAG  
CAGTCTTTCTGAAATAATGTATTAGGTTCTTCCAACCTCG  
TTATATCAGCTAGGCAGGTTTAGAAGTATTTTAGGCTCG  
GCTTAACAACAATAAACTAAAAGT 3'

20 SEQ ID NO: 11 DNA sequence for the internal transcribed spacer of  
*Pichia kluyveri*.

5' CTGTGATTTATATCTTATACACATGCGTGAGCGCACCAA  
ACACCTAAAATTGTAATAATACCAGTCACTAAGTTTTAA  
CAAAACAAAACCTTTCAACAACGGATCTCTTGGTTCTCGC  
25 ATCGATGAAGAGCGCAGcGAAATGCGATACCTAGTGTG  
AATTGCAGCCATCGTGAATCATCGAGTTCTTGAACGCAC  
ATTGCGCCCCATGGTATTCCATGGGGCATGCCTGTCTGA  
GCGTCGTTTCCTTCTTTCGCAAGCAGAGTTGAGAACAG  
GCTATGCCTTTTTTCGAAATGGAACGTCGTGGACGAAGT  
30 GAACTAAACTTTTAGCACGCTTTGGCCGCCGAACTTTA  
ACTAAGC 3'

SEQ ID NO: 12 DNA sequence for the internal transcribed spacer of  
*Candida krusei*.

5' CTGTGATTTACTACTACACTGCGTGAGCGGAACGAAAA  
CAACAACACCTAAAATGTGGAATATAGCATATAGTCGA

5 CAAGAGAAATCTACGAAAAAACAACAAAACCTTTCAAC  
 AACGGATCTCTTGGTTCTCGCATCGATGAAGAGCGCAG  
 CGAAATGCGATACCTAGTGTGAATTGCAGCCATCGTGA  
 ATCATCGAGTTCTTGAACGCACATTGCGCCCTCGGCAT  
 10 TCCGGGGGGGCATGCCTGTTTGAGCGTCGTTTCCATCTTG  
 CGCGTGCGCAGAGTTGGGGGAGCGGAGCGGACGACGTG  
 TAAAGAGCGTCGGAGCTGCGACTCGCCTGAAAGGGGAGC  
 GAAGCTGGCCGAGCGAACTAGACTTTTTTTTCAGGGACG  
 CTTGGCGGCCGAGAGCGAGTGTGCGAGACAACAAAAA  
 GC 3'

SEQ ID NO: 13 DNA sequence for the ITS1 of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.

5' AAGAAATTTAATAATTTTGAAAATGGATTTTTTTGTTTT  
 GGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAGACAA  
 15 GAGATGGAGAGTCCACCCGGGCCTGCGCTTAAGTGCGC  
 GGTCTTGCTAGGCTTGTAAGTTTCTTTCTTGCTATTCCA  
 AACGGTGAGAGATTTCTGTGCTTTTGTTATAGGACAATT  
 AAAACCGTTTCAATACAACACACTGTGGAGTTTTCATAT  
 20 CTTTGCAACTTTTCTTTGGGCATTTCGAGCAATCGGGGC  
 CCAGAGGTAACAAACACAAACAATTTTATCTATTCATTA  
 AATTTTTGTCAAAAACAAGAATTTTCGTAAGTGGAAATT  
 TTAATATATTAA 3'

SEQ ID NO: 14 DNA sequence for the ITS2 of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.

25 5' CCTTCTCAAACATTCTGTTTGGTAGTGAGTGATACTCTT  
 TGGAGTTAACTTGAAATTGCTGGCCTTTTCATTGGATGT  
 TTTTTTTTCCAAAGAGAGGTTTCTCTGCGTGCTTGAGGT  
 ATAATGCAAGTACGGTCGTTTTAGGTTTTACCAACTGCG  
 30 GCTAATCTTTTTTTATACTGAGCGTATTGGAACGTTATC  
 GATAAGAAGAGAGCGTCTAGGCGAACAATGTTCTTAA  
 GT 3'

SEQ ID NO: 15 DNA sequence for the ITS1 of *Saccharomycodes ludwigii*.

5' AAGAAAAAACTGTTTATAAACAGACGGTAGACTTTTC  
TTGGGGAGAGGTTGTTGATTGCTTTGGCCTGCGCTTAAC  
TGC GCGGCTAGTAGTTGATGATTTTGTATTATCCGAGA  
CGAAGGAAACGTCTGATTTTAAAAACATTATACACTTTG  
5 GAGTACTTTTTTTAATGTATTTCTTCCTTGGACGAGCAA  
TTGTTCAAGGGTCAATAAACACAAACAATTTTTTTTTTA  
TTTAAATTTAAATAATTCAAAATATATCATTCTTTTA  
TTAGGAATATAAAAATTTTA 3'

10 SEQ ID NO: 16 DNA sequence for the ITS2 of *Saccharomyces ludwigii*.

5' CCTTCTCAAAAGAGTTTTTTTATTCTTTTGGTTGTGAGTG  
ATACTCTTTCCTTTTACAGGGAAGGGGTAACTTGAAAT  
TGTTGCCTAGCAAAGAAGAATTTTGATTGAAATTTCTTG  
15 TTTATTACTATTAGGTTTATCCCACTAGTGATTATTGA  
GAGTTTTTATTACAGAGTCTTTTCACTTGCTATAATACT  
ATTCTATAAGT 3'

SEQ ID NO: 17 DNA sequence for the ITS1 of *Dekkera bruxellensis*.

20 5' CAGGATGCTGGGCGCAAGCCCGTGCAGACACGTGGATA  
AGCAAGGATAAAAATACATTAAATTTATTTAGTTTAGTC  
AAGAAAGAATTTTA 3'

SEQ ID NO: 18 DNA sequence for the ITS2 of *Dekkera bruxellensis*.

25 5' CCTTCTCACTATTTAGTGGTTATGAGATTACACGAGGGT  
GTTTTCTTCAAAGGAAAGAGGGGAGAGTGAGGGGATAA  
TGATTTAAGGTTTCGGCCGTTTATTATTTTTTCTTCTCC  
CCCAGTTATCAAGT 3'

SEQ ID NO: 19 DNA sequence for the ITS1 of *Dekkera intermedia*.

30 5' CAGGATGCTGGGCGCAAGCCCGTGCAGACACGTGGATA  
AGTAAGGATAAAAATACATTAAATTTATTTAGTTTATAGT  
CAAGAAAGAATTTTA 3'

SEQ ID NO: 20 DNA sequence for the ITS2 of *Dekkera intermedia*.

5' CCTTCTCACTATTTAGTGGTTATGAGATTACACGAGGGT  
GTTTTCTTCAAAGGAAAGAGGGGAGAGTGAGGGGATAA  
TGATTTAAGGTTTCGGCCGTTTCATTATTTTTTCTTCTCCC  
5 CCAGTTATCAAGT 3'

SEQ ID NO: 21 DNA sequence for the ITS1 of *Botrytis cinerea*.

5' CAGAGTTCATGCCCCGAAAGGGTAGACCTCCCACCCTTG  
TGTATTATTACTTTGTTGCTTTGGCGAGCTGCTTTCGGG  
CCTTGTATGCTCGCCAGAGAATAACCAAACTCTTTTAT  
10 TAATGTCGTCTGAGTACTATATAATAGTTA 3'

SEQ ID NO: 22 DNA sequence for the ITS2 of *Botrytis cinerea*.

5' CAACCCTCAAGCTTAGCTTGGTATTGAGTCTATGTCAGT  
AATGGCAGGCTCTAAAATCAGTGGCGGCCGCTGGGT  
CCTGAACGTAGTAATATCTCTCGTTACAGGTTCTCGGTG  
15 TGCTTCTGCCAAAACCCAAATTITTTCTATGG 3'

SEQ ID NO: 23 DNA sequence for the ITS1 of *Penicillium crustosum*.

5' CCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGTG  
TTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAAC  
GGCCGCCGGGGGGCTTACGCCCCCGGGCCCGCGCCCGC  
20 CGAAGACACCCTCGAACTCTGTCTGAAGATTGAAGTCT  
GAGTGAAAATATAAATTATTTA 3'

SEQ ID NO: 24 DNA sequence for the ITS2 of *Penicillium crustosum*.

5' CTGCCCTCAAGCCCGGCTTGTTGTTGGGCCCCGTCCCC  
CGATCTCCGGGGGACGGGCCCCGAAAGGCAGCGGCGGCA  
CCGCGTCCGGTCTCGAGCGTATGGGGCTTTGTCACCCG  
25 CTCTGTAGGCCCGGCGGCGCTTGCCGATCAACCCAAA  
TTTTTATCCAGG 3'

SEQ ID NO: 25 DNA sequence for the ITS1 of *Penicillium expansum*.

5' CCGAGTGAGGGGCCCTTTGGGTCCAACCTCCCACCCGTGT  
TTATTACCTCGTTGCTTCGGCGGGCCCGCCTTAACTGG  
CCGCCGGGGGGCTCACGCCCCCGGGCCCGCGCCCGCCG  
AAGACACCCCCGAACTCTGCCTGAAGATTGTCGTCTGA  
GTGAAAATATAAATTATTTA 3'

SEQ ID NO: 26 DNA sequence for the ITS2 of *Penicillium crustosum*.

5' CTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCCCGTCCTC  
CGATTCCGGGGGACGGGCCCCGAAAGGCAGCGGCGGCAC  
CGCGTCCGGTCTCGAGCGTATGGGGCTTTGTCACCCGC  
TCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAATT  
TTATCCAGG 3'

SEQ ID NO: 27 DNA sequence for the ITS1 of *Hanseniaspora guilliermondii*.

5' GATTGAATTATCATTGTTGCTCGAGTTCTAGTTTTAGAT  
CTTTTACAATAATGTGTATCTTTATTGAAGATGTGCGCT  
TAATTGCGCTGCTTTTTTAAAGTGTCGCAGTAGAAGTAA  
TCTTGCTTGAATCTCAGTCAACGTTTACACACATTGGAG  
TTTTTTTACTTTAATTTAATTCTTTCTGCTTTGAATCGAA  
AGGTTCAAGGCAAAAAACAAACACAAACAATTTTATTT  
TATTATAATTTTTTAACTAAACCAAATTCCTAACGGA  
AATTTTAAAATAATTTA 3'

SEQ ID NO: 28 DNA sequence for the ITS2 of *Hanseniaspora guilliermondii*.

5' CCTTCTCAAAAGATAATTTTTTATTTTTTGGTTGTGGGC  
GATACTCAGGGTTAGCTTGAAATTGAAGATTGTTTCAAT  
CTTTTTTAATTCAACACTTAGCTTCTTTGGAGACGCTGT  
TCTCGCTGTGATGTATTTATGAATTTATTCGTTTTACTTT  
ACAAGGGAAATGGTAATGTACCTTAGGCAAAGGGTTGC  
TTTAATATTCATCAAGT 3'

30 SEQ ID NO: 29 DNA sequence for the ITS1 of *Debaryomyces carsonii*.

5' CAGTATTCTTTTTGCCAGCGCTTAACTGCGCGGCGAAAT  
AAACCTTACACACAATGTTTTTTGTTATTACAGGAACCTT



TTGCTTTGGCTTGTCTCTAGAAATAGAGTTGGGCCAAAG  
GTTTAACTAACTTCAATTTATTTGAACTATTTTCTTAT  
TGAAATGTCACCTTGTGATTAAATTCAAAAAATCTTCA  
3'

5 SEQ ID NO: 30 DNA sequence for the ITS2 of *Debaryomyces carsonii*.

5' CTCTCTCAAACCTTAGGGTTTGGTATTGAGTGATACTCT  
TAGTCGAACTAGGCGTTTGCTTGAAATGTATCGGCATGA  
GTGGTACTAGATTAGTGCTTCAGATTTTCAATGTATTA  
GGTTTATCCAACCTCGTTGAATAGTCTGATGGCAAGTGTT  
10 TAGTAACTATGGCTCGGCCTAACAACAACAACAAGT  
3'

SEQ ID NO: 31 DNA sequence for the ITS1 of *Pichia anomala*.

5' TAGTATTCTATTGCCAGCGCTTAATTGCGCGGCGATAAA  
CCTTACACACATTGTCTAGTTTTTTTGAACCTTTGCTTTGG  
15 GTGGTGAGCCTGGCTTACTGCCCAAAGGTCTAAACACA  
TTTTTTTAATGTTAAAACCTTTAACCAATAGTCATGAAA  
ATTTTAAACAAAATTAAAATCTTCA 3'

SEQ ID NO: 32 DNA sequence for the ITS2 of *Pichia anomala*.

5' CTCTCTCAAACCTTCGGGTTTGGTATTGAGTGATACTCT  
20 GTCAAGGGTTAACTTGAAATATTGACTTAGCAAGAGTG  
TACTAATAAGCAGTCTTTCTGAAATAATGTATTAGGTTC  
TTCCAACCTCGTTATATCAGCTAGGCAGGTTTAGAAGTAT  
TTTAGGCTCGGCTTAACAACAATAAACTAAAAGT 3'

SEQ ID NO: 33 DNA sequence for the ITS1 of *Pichia kluyveri*.

5' CTGTGATTTATATCTTATACACATGCGTGAGCGCACCAA  
25 ACACCTAAAAATTGTAATAATACCAGTCACTAAGTTTAA  
CAAAACA 3'

SEQ ID NO: 34 DNA sequence for the ITS2 of *Pichia kluyveri*.

5' CCTTCTTGCGCAAGCAGAGTTGAGAACAGGCTATGCCTT  
TTTCGAAATGGAACGTCGTGGACGAAGTGAAGTAACT  
TTAGCACGCTTTGGCCGCCGAACTTTAACTAAGC 3'

SEQ ID NO: 35 DNA sequence for the ITS1 of *Candida krusei*.

5' CTGTGATTTACTACTACTGCGTGAGCGGAACGAAAA  
CAACAACACCTAAAATGTGGAATATAGCATATAGTCGA  
CAAGAGAAATCTACGAAAAAACAACA 3'

SEQ ID NO: 36 DNA sequence for the ITS2 of *Candida krusei*.

10 5' CCATCTTGCGCGTGCGCAGAGTTGGGGGAGCGGAGCGG  
ACGACGTGTAAAGAGCGTCGGAGCTGCGACTCGCCTGA  
AAGGGAGCGAAGCTGGCCGAGCGAACTAGACTTTTTTT  
CAGGGACGCTTGGCGCCGAGAGCGAGTGTTGCGAGAC  
AACAAAAAGC 3'

SEQ ID NO: 37 Oligonucleotide Sequence SXUITS1a.

15 5' TTTTGGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAG  
ACAAGAGATGGAGAGTCCACCC 3'

SEQ ID NO: 38 Oligonucleotide Sequence SXLITS2a.

5' CCGTACTTGCATTATACCTCAAGCACGCAGAGAAACCT  
CTCTTTGGAAAAAAAAACA 3'

20 SEQ ID NO: 39 Oligonucleotide Sequence SXUITS1b.

5' TGGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAGACA  
AGAGATGGAGAGTCCACCCGGG 3'

SEQ ID NO: 40 Oligonucleotide Sequence SXLITS2b.

25 5' CGACCGTACTTGCATTATACCTCAAGCACGCAGAGAAA  
CCTCTCTTTGGAAAAAAAAAAC 3'

SEQ ID NO: 41 Oligonucleotide Sequence SLITS1a.

5' CTGTTTATAAACAGACGGTAGACTTTTCTTGGGGAGAGG  
TTGTTGATTGCTTTGGCCTGCGCTTAAGTGCAGCGGCTAG  
TAGTTGATGATTTTGTATTATCCGAGACGAA 3'

5 SEQ ID NO: 42 Oligonucleotide Sequence SLLITS2a.

5' TAATAAAACTCTCAATAATCACTAGTTGGGATAAACC  
TAATAGTAATAACAAGAAATT 3'

SEQ ID NO: 43 Oligonucleotide Sequence SLITS1b.

10 5' TTTATAAACAGACGGTAGACTTTTCTTGGGGAGAGGTTG  
TTGATTGCTTTGGCCTG 3'

SEQ ID NO: 44 Oligonucleotide Sequence SLLITS2b.

5' CTGTAATAAAACTCTCAATAATCACTAGTTGGGATAA  
ACCTAATAGTAATAACAAGAAAT 3'

SEQ ID NO: 45 Oligonucleotide Sequence BRUITS1a

15 5' GGTGAACCTGCGGAAGGATCATTACAGGATGCTGGGCG  
CAAGCCCGTGCAGACACGTGGATAAGCAAGGATAAAAA  
TACAT 3'

SEQ ID NO: 46 Oligonucleotide Sequence BRLITS2a.

20 5' ACTGGGGGAGAAGAAAAAATAATGAACGGCCGAAAC  
CTTAAATCATTATCCCCTCACTCTCCCCTCTTCCTTTGA  
AGAAAACACCCTCGTGTAATCTCATAACCACTA 3'

SEQ ID NO: 47 Oligonucleotide Sequence BRUITS1b.

5' GGTGAACCTGCGGAAGGATCATTACAGGATGCTGGGCG  
CAAGCCCGTGCAGACACG 3'

SEQ ID NO: 48      Oligonucleotide Sequence BRLITS2b.

5'      AACTGGGGGAGAAGAAAAATAATGAACGGCCGAAAC  
CTTAAATCATTATCCCCTCACTCTCCCCTCTTTCCTTTGA  
AGAAAACACCCTCGTGTAATCTCATAACCACTA 3'

5      SEQ ID NO: 49      Oligonucleotide Sequence BCUITS1a.

5'      TGGCGAGCTGCTTTCGGGCCTTGTATGCTCGCCAGAGAA  
TACCAAACTCTTTTAT 3'

SEQ ID NO: 50      Oligonucleotide Sequence BCLITS2.

10      5'      GCGCCGCCACTGATTTTAGAGCCTGCCATTACTGACATA  
GACTCAATACCAAGCTAA 3'

SEQ ID NO: 51      Oligonucleotide Sequence BCUITS1b.

5'      GCTGCTTTCGGGCCTTGTATGCTCGCCAGAGAATACCAA  
AACTCTTTTATTAATGT 3'

SEQ ID NO: 52      Oligonucleotide Sequence PXUITS1a.

15      5'      CCCTYTGGGTCCAACCTCCCACCCGTCTTTATTTACCT  
TGTTGCTTCGGCGGGCCCGCC 3'

SEQ ID NO: 53      Oligonucleotide Sequence PXLITS2a.

5'      TCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAA  
AGCCCCATACGCTCGAGGACCGGACGCGGTGC 3'

20      SEQ ID NO: 54      Oligonucleotide Sequence PXUITS1b.

5'      CCCTYTGGGTCCAACCTCCCACCCGTCTTTATTTACCTC  
GTTGCTTCGGCGGGCCCGC 3'

SEQ ID NO: 55 Oligonucleotide Sequence PXLITS2b.

5' AGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCC  
ATACGCTCGAGGACCGG 3'

SEQ ID NO: 56 Oligonucleotide Sequence HGUTS1.

5 AATCTTGCTTGAATCTCAGTCAACGTTTACACACATTGG  
AGTTTTTTTACTTTAATTTAATTCTTTCTGCTTTGAATCG  
AAAGGTTCAAGGCAAAAAC 3'

SEQ ID NO: 57 Oligonucleotide Sequence HGLITS2.

10 5' TAAATACATCACAGCGAGAACAGCGTCTCCAAAGAAGC  
TAAGTGTTGAATTA AAAAAGATT 3'

SEQ ID NO: 58 Oligonucleotide Sequence DXLITS2a.

5' CTCATGCCGATACATTTCAAGCAAACGCCTAGTTCGACT  
AAGAGTATCACTCAATACCAA 3'

SEQ ID NO: 59 Oligonucleotide Sequence DXLITS2b.

15 5' TAAACACTTGCCATCAGACTATTCAACGAGTTGGATAA  
ACCTAATACATTGAAAAATCTGA 3'

SEQ ID NO: 60 Oligonucleotide Sequence PAUTS1.

5' TTGCTTTGGGTGGTGAGCCTGGCTTACTGCCCAAAGGTC  
TAAACACATTTTTTTAATGT 3'

20 SEQ ID NO: 61 Oligonucleotide Sequence PALITS2.

5' ATACATTATTTTCAGAAAGACTGCTTATTAGTACACTCTT  
GCTAAGTCAATATTTCAAGTTA 3'

SEQ ID NO: 62 Oligonucleotide Sequence PKLITS2.

5' GCGGCCAAAGCGTGCTAAAAGTTTAGTTCACTTCGTCCA  
CGACGTTCCATTTCGAAAAAGG 3'

SEQ ID NO: 63 Oligonucleotide Sequence CKUITS1.

5 5' GCGGAACGAAAACAACAACACCTAAAATGTGGAATATA  
GCATATAGTCGACAAGAGAAATC 3'

SEQ ID NO: 64 Oligonucleotide Sequence CKLITS2.

5' TCAGGCGAGTCGCAGCTCCGACGCTCTTTACACGTCGTC  
CGCTCCGCTCCCCCACTCT 3'

10 SEQ ID NO: 65 Oligonucleotide Sequence SXU99.

5' CTTTACTGGGCAAGAAGAC 3'

SEQ ID NO: 66 Oligonucleotide Sequence SXL658.

5' AAGCACGCAGAGAAACC 3'

SEQ ID NO: 67 Oligonucleotide Sequence SXU102.

15 5' TTACTGGGCAAGAAGACAAG 3'

SEQ ID NO: 68 Oligonucleotide Sequence SXL661.

5' CTCAAGCACGCAGAGAA 3'

SEQ ID NO: 69 Oligonucleotide Sequence SLU85.

5' GACTTTTCTTGGGGAGAG 3'

SEQ ID NO: 70 Oligonucleotide Sequence SLL635.

5' TCACTAGTTGGGATAAACCT 3'

SEQ ID NO: 71 Oligonucleotide Sequence SLU88.

5' TTTTCTTGGGGAGAGG 3'

5 SEQ ID NO: 72 Oligonucleotide Sequence SLL636.

5' ATCACTAGTTGGGATAAACC 3'

SEQ ID NO: 73 Oligonucleotide Sequence SLU136.

5' CGGCTAGTAGTTGATGATT 3'

SEQ ID NO: 74 Oligonucleotide Sequence SLL634.

10 5' CACTAGTTGGGATAAACCTA 3'

SEQ ID NO: 75 Oligonucleotide Sequence BRU53A.

5' ATTACAGGATGCTGGGC 3'

SEQ ID NO: 76 Oligonucleotide Sequence BRU53B.

5' ATTACAGGATGCTGGG 3'

15 SEQ ID NO: 77 Oligonucleotide Sequence BRU77.

5' CGTGCAGACACGTGGAT 3'

SEQ ID NO: 78 Oligonucleotide Sequence BRL339.

5' CTTTGAAGAAAACACCCTC 3'

SEQ ID NO: 79 Oligonucleotide Sequence BRL367.

5' AATCATTATCCCCTCACTC 3'

SEQ ID NO: 80 Oligonucleotide Sequence BRL390.

5' TAATGAACGGCCGAAAC 3'

5 SEQ ID NO: 81 Oligonucleotide Sequence BCU136.

5' TTGTATGCTCGCCAGAG 3'

SEQ ID NO: 82 Oligonucleotide Sequence BCL393.

5' GCCTGCCATTACTGACA 3'

SEQ ID NO: 83 Oligonucleotide Sequence BCU142.

10 5' GCTCGCCAGAGAATACC 3'

SEQ ID NO: 84 Oligonucleotide Sequence PXU87.

5' ACCCGTCTTTATTTTACCTT 3'

SEQ ID NO: 85 Oligonucleotide Sequence PXL495.

5' CTACAGAGCGGGTGACA 3'

15 SEQ ID NO: 86 Oligonucleotide Sequence PXU86.

5' CACCCGTGTTTATTTTACCT 3'

SEQ ID NO: 87 Oligonucleotide Sequence PXL482.

5' CAAAGCCCCATACGC 3'



SEQ ID NO: 88 Oligonucleotide Sequence PXL491.

5' AGCGGGTGACAAAGC 3'

SEQ ID NO: 89 Oligonucleotide Sequence HGU193.

5' CAACGTTTACACACATTGG 3'

5 SEQ ID NO: 90 Oligonucleotide Sequence HGU231.

5' AATTCTTTCTGCTTTGAATCG 3'

SEQ ID NO: 91 Oligonucleotide Sequence HGL601.

5' CAGCGTCTCCAAAGAAGCTAA 3'

SEQ ID NO: 92 Oligonucleotide Sequence DXL447.

10 5' GCAAACGCCTAGTTCGACTAA 3'

SEQ ID NO: 93 Oligonucleotide Sequence DXL526.

5' ATTCAACGAGTTGGATAAACC 3'

SEQ ID NO: 94 Oligonucleotide Sequence PAU133.

5' GGCTTACTGCCCAAAGGTC 3'

15 SEQ ID NO: 95 Oligonucleotide Sequence PAL451.

5' TGCTTATTAGTACACTCTTGC 3'

SEQ ID NO: 96 Oligonucleotide Sequence PKL356.

5' GTTTAGTTCACCTTCGTCCACG 3'

SEQ ID NO: 97     Oligonucleotide Sequence CKU104.

5'     CCTAAAATGTGGAATATAGCA 3'

SEQ ID NO: 98     Oligonucleotide Sequence CKL354.

5'     ACGCTCTTTACACGTCGTC 3'

5     SEQ ID NO: 99     Oligonucleotide Sequence ITS5.

5'     GGAAGTAAAAGTCGTAACAAGG 3'

SEQ ID NO: 100     Oligonucleotide Sequence ITS4.

5'     TCCTCCGCTTATTGATATGC 3'